

WHAT IS CLAIMED IS:

1. A method of monitoring a flow rate of a fluidic material in a microfluidic device, the method comprising:

- (i) flowing a first marker moiety through the at least one microscale channel;
- (ii) flowing the fluidic material through the at least one microscale channel;
- (iii) flowing a second marker moiety through the at least one microscale channel;
- (iv) detecting the first marker moiety, resulting in detection of a first signal having, a first area and a first retention time;
- (v) detecting the second marker moiety, resulting in detection of a second signal having a second area and a second retention time; and,
- (vi) deconvoluting the first signal and the second signal to provide an indication of the flow rate of the fluidic material, wherein the deconvoluting comprises identifying differences between two or more of: the first signal, the second signal, a first selected standard, or a second selected standard, thereby monitoring the flow rate of the fluidic material.

2. The method of claim 1, wherein the deconvoluting step comprises comparing the first selected standard or the second selected standard to one or more of: the first retention time, the first area, the second area, and the second retention time, which first selected standard has a first selected area and a first selected retention time and, which second selected standard has a second selected area and a second selected retention time.

3. The method of claim 1, wherein deconvoluting comprises identifying or quantifying differences between two or more of: the first signal, the second signal, the first selected standard, or the second selected standard, which differences comprises differences in one or more of: height, width, area under the curve, width at half-maximum height, or retention time.

4. The method of claim 1, wherein the first selected standard and second selected standard comprise initial assay conditions and deconvoluting comprises identifying and quantifying changes in the first and second signal from the initial assay conditions.

5. The method of claim 2, wherein comparing comprises identifying differences between one or more of: the first area and the first selected area, the second area and the second selected area, the first retention time and the first selected retention time, and the second retention time and the second selected retention time.

6. The method of claim 2, wherein the first selected retention time and the second selected retention time are different.

10 7. The method of claim 1, further comprising modulating the flow rate of the fluidic material, wherein the modulating comprises one or more of:

- (a) altering flow of the fluidic material through the at least one microscale channel;
- (b) altering flow of the first marker moiety through the at least one microscale channel; and,
- (c) altering flow of the second marker moiety through the at least one microscale channel.

15 8. The method of claim 1, further comprising altering the flow rate of the fluidic material into the at least one microscale channel in response to the indication of the flow rate provided by step (vi).

20 9. The method of claim 1, further comprising altering the flow rate of the fluidic material in the at least one microscale channel in the event that the first area and the second area are different.

10. The method of claim 9, further comprising increasing the flow rate in the event that the first area is larger than the second area.

25 11. The method of claim 9, further comprising decreasing the flow rate in the event that the first area is smaller than the second area.

12. The method of claim 8, further comprising increasing the flow rate in the event that the first area or the second area is smaller than a selected standard area.

13. The method of claim 8, further comprising decreasing the flow rate in the event that the first area or the second area is larger than a selected standard area.

5           14. The method of claim 8, further comprising changing the flow rate in the event that the first area and the second area have increased or decreased.

10           15. The method of claim 14, wherein deconvoluting comprises identifying a change in the ratio of one or more of: peak heights, peak widths, or peak areas, for the first signal and the second signal, thereby determining whether the flow rate has increased or decreased.

16. The method of claim 8, further comprising increasing the flow rate in the event that the first retention time or the second retention time is longer than a selected standard retention time.

15           17. The method of claim 8, further comprising decreasing the flow rate in the event that the first retention time or the second retention time is shorter than a selected standard retention time.

18. The method of claim 1, further comprising providing a microfluidic device, the device comprising:

20           (a) a reaction channel; and,  
              (b) a separation channel, which separation channel intersects the reaction channel.

19. The method of claim 18, comprising inducing flow of the fluidic material in the reaction channel by applying pressure to the reaction channel and electrokinetically inducing flow of the fluidic material in the separation channel.

25           20. The method of claim 19, wherein the first area and the second area provide an indication of the flow rate in the reaction channel and the first retention time and the second retention time provide an indication of the flow rate in the separation channel.

21. The method of claim 19, further comprising increasing the pressure applied in the reaction channel in the event that the first area or the second area decreases in comparison to a selected standard area.

22. The method of claim 19, further comprising decreasing the pressure applied in the reaction channel in the event that the first area or the second area increases in comparison to a selected standard area.

23. The method of claim 19, further comprising altering the pressure applied in the reaction channel in the event that the first area and the second area are different.

24. The method of claim 23, comprising increasing the pressure applied in the reaction channel in the event that the first area is larger than the second area.

25. The method of claim 23, comprising decreasing the pressure applied in the reaction channel in the event that the first area is smaller than the second area.

26. The method of claim 19, wherein electrokinetically inducing flow comprises applying a voltage gradient across the separation channel.

27. The method of claim 26, further comprising increasing the voltage applied across the separation channel in the event that the first retention time or the second retention time is longer than a first selected standard retention time or a second selected standard retention time.

28. The method of claim 26, further comprising decreasing the voltage applied across the separation channel in the event that the first retention time or the second retention time is shorter than a first selected standard retention time or a second selected standard retention time.

29. The method of claim 1, further comprising performing steps (ii), (iii) and (iv) such that the fluidic material is flowed after the first marker moiety and prior to the second marker moiety.

30. The method of claim 1, wherein the fluidic material comprises a label moiety.

PCT  
10  
15  
20

31. The method of claim **30**, wherein the label moiety is a fluorescent moiety.
32. The method of claim **1**, wherein the first marker moiety is a non-reactive moiety.
33. The method of claim **1**, wherein the first marker moiety comprises a label moiety.
34. The method of claim **33**, wherein the label moiety is a fluorescent moiety.
35. The method of claim **1**, wherein the second marker moiety is a non-reactive moiety.
36. The method of claim **1**, wherein the second marker moiety comprises a label moiety.
37. The method of claim **35**, wherein the label moiety comprises a fluorescent moiety.
38. The method of claim **1**, wherein the first marker moiety and the second marker moiety are the same.
39. The method of claim **1**, wherein the first marker moiety and the second marker moiety are different.
40. The method of claim **39**, wherein the first marker moiety and the second marker moiety have different electrophoretic mobilities.
41. The method of claim **39**, wherein the first retention time and the second retention time are different.
42. The method of claim **40**, wherein the first marker moiety is neutral and the second marker moiety is charged.
43. The method of claim **40**, wherein the first marker moiety is charged and the second marker moiety is neutral.

44. The method of claim 1, wherein the first marker moiety and the second marker moiety comprise a first fluorescent moiety and a second fluorescent moiety.

45. The method of claim 44, wherein detecting comprises fluoresently detecting the first fluorescent moiety and the second fluorescent moiety.

5 46. The method of claim 47, further comprising detecting the fluidic material, the first marker moiety and the second marker moiety with a single detector.

47. The method of claim 1, the method further comprising detecting the fluidic material, resulting in detection of at least a third signal.

48. The method of claim 1, further comprising iteratively repeating one or more of: steps (i) through (vi).

50 49. The method of claim 1, further comprising providing a fluid direction system operably coupled to the microfluidic device, which fluid direction system directs one or more of:

- (a) flow of the fluidic material through the at least one microscale channel;
- (b) flow of the first marker moiety through the at least one microscale channel; and,
- (c) flow of the second marker moiety through the at least one microscale channel.

55 50. The method of claim 49, further comprising providing a detection system, which detection system comprises a detector that detects one or more of: the first signal and the second signal.

51. The method of claim 50, further comprising providing a computer, operably coupled to the detection system and the fluid direction system, the computer comprising software, which software comprises at least a first instruction set, which first instruction set instructs the fluid direction system to modulate the flow rate of the fluidic material in response to one or more of: the first signal and the second signal detected by the detection system.

52. The method of claim 51, wherein the first instruction set deconvolutes one or 25 more of: the first signal and the second signal, to provide an indication of the flow rate of the fluidic material.

53. The method of claim 50, wherein the first instruction set determines a difference between one or more of: a first selected standard and the first signal, a second selected standard and the second signal, and the first signal and the second signal and instructs the fluid direction system to modulate the flow of the fluidic material in the at least one microscale channel based on the difference.

54. The method of claim 50, wherein the software comprises a second instruction set, which second instruction set calculates the flow rate of the fluidic material.

55. The method of claim 1, further comprising:

(vii) providing a pressure source and a sample source, which pressure source is fluidly

10 coupled to the microfluidic device, and

(viii) introducing the fluidic material into the microfluidic device from the sample source by drawing fluid from the sample source into the microfluidic device by applying pressure from the pressure source.

56. The method of claim 55, wherein the pressure source comprises a siphon, a vacuum source, a programmable syringe pump, or an electroosmotic pump, which siphon, vacuum source, programmable syringe pump, or electroosmotic pump introduces the fluidic material into the microfluidic device from the sample source.

57. The method of claim 55, wherein the sample source comprises a plurality of sample sources and a plurality of marker sources.

20 58. The method of claim 57, wherein the sample source further comprises a plurality of buffer sources.

59. The method of claim 57, further comprising introducing the first marker moiety, the second marker moiety, or the first marker moiety and the second marker moiety, from the sample source into the microfluidic device after introducing the fluidic material.

25 60. The method of claim 57, further comprising introducing the first marker moiety into the microfluidic device before introducing the fluidic material and introducing the second marker moiety into the microfluidic device after introducing the fluidic material.

5           **61.** The method of claim **57**, further comprising introducing a plurality of fluidic materials from the sample source into the at least one microscale channel.

10           **62.** The method of claim **61**, wherein the plurality of fluidic materials comprises about 96 or more, about 384 or more, or about 1536 or more different fluidic materials.

15           **63.** The method of claim **61**, further comprising introducing the first marker moiety, the second marker moiety, or the first marker moiety and the second marker moiety, into the at least one microscale channel from the sample source after introducing each member of the plurality of fluidic materials.

20           **64.** The method of claim **61**, further comprising introducing the first marker moiety prior to introducing each member of the plurality of fluidic materials and introducing the second marker moiety after introducing each member of the plurality of fluidic materials.

25           **65.** The method of claim **62**, further comprising introducing the first marker moiety, the second marker moiety, or the first marker moiety and the second marker moiety into the at least one microscale channel from the sample source after introducing about five or more members of the plurality of fluidic materials into the at least one microscale channel.

30           **66.** The method of claim **62**, further comprising introducing the first marker moiety prior to introducing about five or more members of the plurality of fluidic materials and introducing the second marker moiety after introducing about five or more members of the plurality of fluidic materials into the at least one microscale channel.

35           **67.** The method of claim **62**, further comprising introducing the first marker moiety, the second marker moiety, or the first marker moiety and the second marker moiety into the at least one microscale channel from the sample source after introducing about ten or more members of the plurality of fluidic materials into the at least one microscale channel.

40           **68.** The method of claim **62**, further comprising introducing the first marker moiety prior to introducing about ten or more members of the plurality of fluidic materials and introducing the second marker moiety after introducing about ten or more member of the plurality of fluidic materials into the at least one microscale channel.

69. The method of claim 62, further comprising introducing the first marker moiety, the second marker moiety, or the first marker moiety and the second marker moiety into the at least one microscale channel from the sample source after introducing about twenty or more members of the plurality of fluidic materials into the at least one microscale channel.

5        70. The method of claim 62, further comprising introducing the first marker moiety prior to introducing about twenty or more members of the plurality of fluidic materials and introducing the second marker moiety after introducing about twenty or more members of the plurality of fluidic materials into the at least one microscale channel.

10      71. A system for monitoring flow rate of a sample in a microfluidic device, the system comprising:

- (i) a microfluidic device comprising a body structure having a plurality of microscale channels disposed therein;
- (ii) a sample source comprising a plurality of samples, which sample source is fluidly coupled to at least one member of the plurality of microscale channels;
- (iii) a fluid direction system fluidly coupled to the microfluidic device, which fluid direction system directs one or more of:
  - movement of the sample from the sample source into the plurality of microscale channels;
  - movement of a first marker moiety through the plurality of microscale channels;
  - movement of a second marker moiety through the plurality of microscale channels;
- (iv) a detection system positioned proximal to the body structure and operably coupled to the fluid direction system, which detection system detects the first marker moiety, the second marker moiety, and the fluidic material, resulting in a first output signal, a second output signal and at least a third output signal; and,
- (v) a computer operably coupled to the detection system and the fluid direction system, which computer receives the first, second, and third output signals and comprises software, which software comprises at least a first instruction set, which first instruction set instructs the fluid direction system to modulate the flow

rate of the fluidic material in response to one or more of: the first signal, the second signal, and the third signal detected by the detection system.

72. The system of claim 71, wherein the plurality of channels comprises a reaction channel and a separation channel, which separation channel intersects the reaction  
5 channel.

73. The system of claim 72, wherein the fluid direction system comprises a pressure source, which pressure source directs movement of one or more of: the sample, the first marker moiety, and the second marker moiety through the reaction channel, and an electrokinetic source, which electrokinetic source directs movement of one or more of: the sample, the first  
10 marker moiety, and the second marker moiety, through the separation channel.

74. The system of claim 73, wherein the pressure source is a siphon, a vacuum source, a programmable syringe pump, or an electroosmotic pump.

75. The system of claim 73, wherein the first instruction set instructs the fluid direction system to increase the flow rate of the sample in the reaction channel in the event that  
15 the first signal or the second signal has an area that is smaller than a selected standard area.

76. The system of claim 73, wherein the first instruction set instructs the fluid direction system to decrease the flow rate of the sample in the reaction channel in the event that  
the first signal or the second signal has an area that is larger than a selected standard area.

77. The system of claim 73, wherein the first instruction set instructs the fluid  
20 direction system to alter the flow rate of the sample in the reaction channel in the event that the first signal and the second signal have different areas.

78. The system of claim 77, wherein the first instruction set instructs the fluid direction system to increase the flow rate of the sample in the reaction channel in the event that  
the first signal has an area that is larger than the area of the second signal.

25 79. The system of claim 77, wherein the first instruction set instructs the fluid direction system to decrease the flow rate of the sample in the reaction channel in the event that  
the first signal has an area that is smaller than the area of the second signal

80. The system of claim 73, wherein the first instruction set instructs the fluid direction system to increase the flow rate of the sample in the separation channel in the event that the first signal or the second signal has a longer retention time than a selected standard retention time.

5        81. The system of claim 73, wherein the first instruction set instructs the fluid direction system to decrease the flow rate of the sample in the separation channel in the event that the first signal or the second signal has a shorter retention time than a selected standard retention time.

10      82. The system of claim 71, wherein the plurality of microscale channels comprises at least a first channel, which first channel comprises one or more of the first marker moiety and the second marker moiety.

15      83. The system of claim 71, wherein the plurality of microscale channels further comprises a first source and a second source, which first source comprises the first marker moiety and which second source comprises the second marker moiety.

20      84. The system of claim 71, wherein the fluid direction system transports the first marker moiety from the sample source to the microscale channels prior to transporting the sample and transports the second marker moiety from the sample source to the microscale channels after transporting the sample.

25      85. The system of claim 71, wherein the fluid direction system transports a plurality of samples from the sample source to the microfluidic device.

86. The system of claim 85, wherein the plurality of samples comprises about 96 or more, about 384 or more, or about 1536 or more fluidic samples.

25      87. The system of claim 86, wherein the fluid direction system transports the first marker moiety from the sample source to the microfluidic device prior to transporting each member of the plurality of samples and transports the second marker moiety from the sample source to the microfluidic device after transporting each member of the plurality of samples.

88. The system of claim 86, wherein the fluid direction system transports the first marker moiety from the sample source to the microfluidic device prior to transporting about five or more samples and transports the second marker moiety from the sample source to the microfluidic device after transporting about five or more samples.

5        89. The system of claim 86, wherein the fluid direction system transports the first marker moiety from the sample source to the microfluidic device prior to transporting about ten or more samples and transports the second marker moiety from the sample source to the microfluidic device after transporting about ten or more samples.

10      90. The system of claim 86, wherein the fluid direction system transports the first marker moiety from the sample source to the microfluidic device prior to transporting about twenty or more samples and transports the second marker moiety from the sample source to the microfluidic device after transporting about twenty or more samples.

15      91. The system of claim 71, wherein the fluid direction system modulates movement of the sample through the microscale channels in response to one or more of: the first signal, the second signal, and the third signal.

92. The system of claim 71, wherein the detection system comprises a single detector, which detector detects one or more of: the sample, the first marker, and the second marker.

20      93. The system of claim 71, wherein the software further comprises a second instruction set, which second instruction set deconvolutes one or more of: the first signal, the second signal, and the third signal, to provide an indication of the flow rate of the sample.

94. The system of claim 93, wherein the second instruction set comprises instructions for comparing a selected standard to one or more of: the first signal and the second signal.

25      95. The system of claim 93, wherein the second instruction set comprises instructions for comparing the first signal and the second signal.